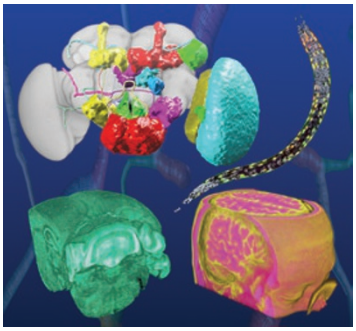
IMAGING AND VISUALIZATION

Connecting the dots in 3D

New software tools help take the pain out of working with huge three-dimensional image datasets and aid in mapping neuronal networks.

Computer-generated three-dimensional (3D) images are finding ever wider use in entertainment, and even scientists are increasingly using 3D images generated from image stacks acquired during serial section imaging of biological samples. Although the surface-rendering methods used in the entertainment industry are fast and mature, "biologists have a lot of image data, which is very different from surface data, and they don't have a very good renderer," says Hanchuan Peng of the Howard Hughes Medical Institute's Janelia Farm Research Campus.

Researchers at Janelia Farm have established an ambitious goal of discovering how the brain works, and an important part of this endeavor is creating a 3D brain atlas. For the fruit fly brain, the first target of Janelia Farm researchers, this is a realistic goal. Individual neurons can be labeled with GFP, and by acquiring fluorescence image stacks through the brain, it should be possible to map all the neurons and their branches.



V3D is being used to visualize and analyze high-dimensional image data from various animal models at different scales including construction of a neuronal wiring atlas of a fruit fly brain. Image courtesy of Hanchuan Peng.

But once investigators have their stacks of images, how do they interact with the data in a convenient way and make quantitative measurements? Existing tools either require the user to step through the individual two-dimensional images and select regions of interest—which is cumbersome and tedious—or use a virtual reality environment requiring specialized equipment to view and interact with a 3D version of the data.

Peng found that when he was trying to construct a 3D atlas of the fruit fly brain derived from tens of thousands of images, the existing tools were inadequate or difficult to use, so he decided to create a new set of tools. The first results of this effort are V3D and V3D-Neuron.

V3D is designed expressly for working with 3D volumetric data and is built on an efficient 3D renderer that allows real-time visualization and manipulation of multi-gigabyte–sized data on a standard computer. Peng and colleagues worked hard to make it user friendly. "With V3D you can drag and drop an image stack and see it in [three dimensions]. If you want to go to anywhere and see more detail, it is just one or two clicks," he says.

The most innovative aspect of the software is how easily users can select visual

SYSTEMS BIOLOGY

TRANSCRIPTION FACTOR INTERACTION MAPS

A systematic map of pair-wise physical interactions among mammalian transcription factors will enable studies of transcriptional control in development and disease.

The specification of cell and tissue types—a question at the heart of biology—depends on patterns of gene expression controlled in part by the activity of transcription factors. It is by now fairly clear that transcription factors do not act alone. Rather, their activity is combinatorial, which is key to understanding their function.

An ambitious step in this direction is described in a recent paper that presents a comprehensive map of physical interactions among mammalian transcription factors. The fruit of a large FANTOM (functional annotation of the mammalian genome) international collaborative effort—including the RIKEN Omics Science Center and the Genome Network Project (GNP) in Japan, the University of California, San Diego in the US, the King Abdullah University for Science and Technology in Saudi Arabia and several other groups around the world—the study reports about 800 pair-wise interactions between transcription factors in mouse and in human.

The researchers used the mammalian two-hybrid system to systematically examine interactions among all mouse or human transcription factors for which cDNA clones expressing full-length proteins could be obtained. "We had collected mouse cDNAs in our previous FANTOM project and human cDNAs in the GNP," points out Harukazu Suzuki, at RIKEN; "those projects were therefore essential for this work." The resulting resource of mapped transcription factor interactions contains many previously unidentified interacting pairs. It is available to other researchers (http://fantom.gsc.riken.jp/4/tf-ppi/) and will undoubtedly spawn many experiments investigating the functional role of these putative interacting pairs in various biological contexts.

But the partnership between RIKEN and other groups did not stop there. "This collaboration brought together the immense data-generation capacity of RIKEN with other expertise, including ours, in systems and network biology," says Trey Ideker at University of California, San Diego and, together with RIKEN's Yoshihide Hayashizaki, the senior author on the paper. The unprecedented transcription factor interaction dataset was ripe for analysis with approaches that Ideker and colleagues had previously developed for network alignment between species or for network-based biomarker discovery.

If transcription factor interactions are important for specifying cell or tissue types in mammalian development, then one might expect that certain interactions would be predictive

features without stereo viewing or virtual reality hardware. There are two options. First, while viewing the 3D image, a user can mouse-click on a region of interest, rotate the image and click on the same region from another angle. The two clicks define rays that intersect at the desired 3D location and create a marker position there. Alternatively, the software will determine the most likely 3D position the user intended to mark using only a single mouse-click by examining the intensity information along the single ray. Peng says that although it is possible to create image data that fools the latter method, with real data the method is surprisingly accurate. And because the user can always rotate the image and quickly adjust the location, it is very fast.

"The most important part of the 3D pinpointing is that once you have the 3D marker information, you can directly measure it or use it as prior knowledge in computer algorithms," says Peng. His team used this approach in their neuron tracing tool V3D-Neuron. Instead of relying on manual tracing or automated tracing followed by manual correction, V3D-Neuron allows the user to quickly pinpoint markers only at the terminals of the neuronal branches in three dimensions, and then an algorithm finds the optimal connecting paths. "This produced much better performance," says Peng.

Work on V3D is continuing. 3D curve drawing has been implemented, other people are designing plugins, and a V3D hackathon is scheduled for this summer at Janelia Farm. It may not be as fun as 3D gaming but V3D promises to make working with 3D image data in the lab much more enjoyable.

Daniel Evanko

RESEARCH PAPERS
Peng, H. *et al.* V3D enables real-time 3D visualization and quantitative analysis of large-scale biological image data sets. *Nat. Biotechnol.* **28**, 348–353 (2010).

of tissue type. In other words, the interactions themselves might function as tissue 'biomarkers'.

To look into this, the researchers classified transcription factor expression profiles from multiple human tissues. They did this either based on quantitative mRNA levels alone or by adding information from the physical interaction network. By focusing on interacting pairs of factors for which the expression levels were highly correlated in one tissue but not in others, they identified interactions that could quite accurately classify tissue type according to embryonic germ layer. "We think this is a major advance of our paper because it shows how protein networks can serve as powerful biomarkers of cell state," says Ideker, though one challenge will be to identify which biomarkers are actually causal for tissue type. Encouragingly, the best predictive network is enriched for homeobox transcription factors, which are known regulators of development.

And the atlas will probably be expanded in the near future, says Suzuki. The researchers are hoping to compare disease transcription factor networks to normal ones, to identify factors involved in these diseases and to pinpoint interactions that may offer novel targets for therapy. As Ideker sums it up, "it's not about the proteins; it's about the networks."

Natalie de Souza

RESEARCH PAPERS
Ravasi, T. *et al.* An atlas of combinatorial transcriptional regulation in mouse and man. *Cell* **140**, 744–752 (2010).

NEWS IN BRIEF

NEUROSCIENCE

Imaging dopamine with MRI

Magnetic resonance imaging (MRI) is a powerful, noninvasive technology for studying the brain. But neurotransmitters such as dopamine have not been directly observed by MRI. Shapiro *et al.* now report the directed evolution of a MRI contrast agent specific for dopamine, based on the heme domain from a bacterial cytochrome. The probe allowed the researchers to image depolarization-triggered dopamine release in live animal brains.

Shapiro, M.G. *et al. Nat. Biotechnol.* **28**, 264–270 (2010).

CHEMICAL BIOLOGY

More potent CALI reagents

Tools for inactivating protein function in cells, chromophore-assisted light inactivation (CALI) reagents, contain a protein-targeting moiety and a chromophore that generates singlet oxygen when irradiated with light. These tools, however, generally suffer from poor targeting efficiency and poor singlet oxygen generation. Lee *et al.* describe highly potent CALI reagents made by tacking a Ru(II)(tris-bipyridyl)$^{2+}$ derivative, a very efficient photocatalyst for generating singlet oxygen, to a highly selective protein-targeting peptoid.

Lee, J. *et al. Nat. Chem. Biol.* **6**, 258–260 (2010).

SPECTROSCOPY

Introducing SHINERS

Surface-enhanced Raman scattering (SERS) is a useful approach for enhancing Raman signals by distributing metal nanoparticles over a surface, but the nanoparticles often stick to each other and to the material being studied. Li *et al.* now introduce shell-isolated, nanoparticle-enhanced Raman spectroscopy, or SHINERS. Gold nanoparticles are coated with an ultrathin alumina or silica shell; the nanoparticles are spread over the surface without sticking, yet they conform to the surface contours and facilitate single-molecule detection.

Li, J.F. *et al. Nature* **464**, 392–395 (2010).

IMAGING AND VISUALIZATION

New red fluorescent proteins

The titanium-sapphire lasers used in two-photon microscopy have low power output in the excitation wavelength range for red fluorescent proteins, limiting their application. Piatkevich *et al.* introduce two new monomeric red fluorescent proteins, named LSS-mKate1 and LSS-mKate2. These proteins have large Stokes shifts, allowing efficient excitation by titanium-sapphire lasers, in addition to high pH stability, photostability and rapid chromophore maturation, making them useful for multicolor intravital imaging.

Piatkevich, K.D. *et al. Proc. Natl. Acad. Sci. USA* **107**, 5369–5374 (2010).

CELL BIOLOGY

Levitating cell cultures

Souza *et al.* describe a method for three-dimensional tissue culture by magnetic cell levitation. The cells are incubated with a hydrogel made up of gold, magnetic iron oxide nanoparticles and bacteriophages. By controlling the magnetic field, the researchers can manipulate the three-dimensional geometry of the cell culture to better represent the *in vivo* tissue structure.

Souza, G.R. *et al. Nat. Nanotechnol.* **5**, 291–296 (2010).